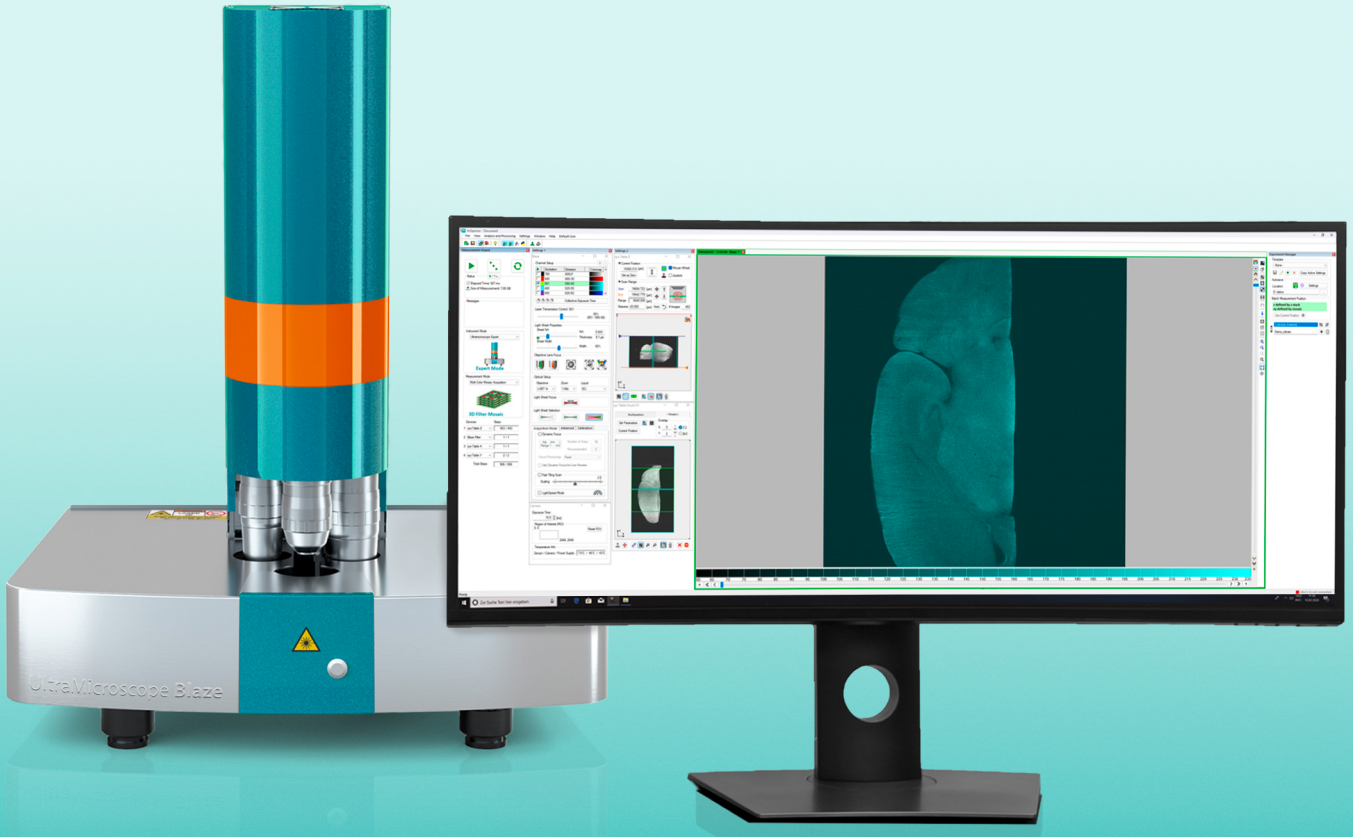
Miltenyi Biotec

ImSpector 8.1

User manual



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ImSpector 8.1

User manual

Original instructions

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1 About this user manual

1.1 Read the user manual first

⚠ WARNING

Before using the ImSpector software, read the safety information in the instrument user manual and all other information contained in this software user manual, including any safety and operating instructions. Pay special attention to all warnings shown on the instrument. Failure to read and follow these instructions could lead to improper or incorrect use and result in damage to the instrument. Improper use could also cause severe personal injury, death, unpredictable results, instrument malfunction, and premature wear of components, shortening the lifetime of the instrument. Any failure to follow these instructions may void your warranty. Keep the user manual and any other related safety and operating instructions provided in a safe place accessible to all users for future reference.

If you have a serious concern regarding the safe use of your instrument, contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Technical Support.

1.2 Presentational conventions

The following text styles are used to indicate specific information types in this user manual.

1.2.1 Text styles

Colored text with colored background	Indicates an important note or hint which is not safety-related
• Text with a bullet	Indicates a list item
Bold text	Indicates software elements, for example, a button, tab, or window of the user interface
`This font`	Indicates code
See **Text styles on page 7**	References to the chapter or section **Text styles** on page 7
See **Figure/Table 4.2**	References to the second figure/table in chapter 4

1.2.2 Step-by-step instructions

1 This is an instructional step.

This is a result.

2 Introduction

2.1 About the software

The ImSpector software is a Windows program for setting up the image acquisition with the UltraMicroscope Blaze Imaging System and the UltraMicroscope Choros Imaging System. The software also provides data visualization and basic analysis functions. The integrated Python scripting package accesses imaging data and microscope devices. Python supports advanced measurement workflows and imaging data analysis.

2.2 Intended use

The ImSpector software in this version is designed for the use with the UltraMicroscope Blaze Imaging System and the UltraMicroscope Choros Imaging System. The software controls the instrument and generates high-resolution images.

The ImSpector software is for research use only in a laboratory environment. Only laboratory professional users may use the software.

3 Basic functions

This chapter describes the basic functions used to operate the instrument. It explains how to switch the instrument on and off. Observing these instructions helps ensure correct operation.

3.1 Switching on the instrument

1. Ensure that all components are connected to the power supply.
2. Switch on the external laser module. The key switch is located on the laser module. Refer to the manufacturer documentation.
3. **CAUTION! Crushing of hands hazard. Keep a safe distance from the instrument after switching on.** Switch on the instrument. The power switch is located on the rear side of the instrument. Refer to the instrument user manual.

   The xyz-Table performs a reference run.
4. Switch on the acquisition workstation, and sign in to the Windows system.
5. Open the ImSpector software. The switched on devices are registered and checked.

3.2 Switching off the instrument

1. Close the ImSpector software.
2. Shut down the Windows system.
3. Switch off the instrument.
4. Switch off the external laser module.

4 Graphical user interface

This chapter describes the graphical user interface. Most functions can be accessed via the menu bar (1). Frequently used functions can also be accessed directly from the toolbar (2).

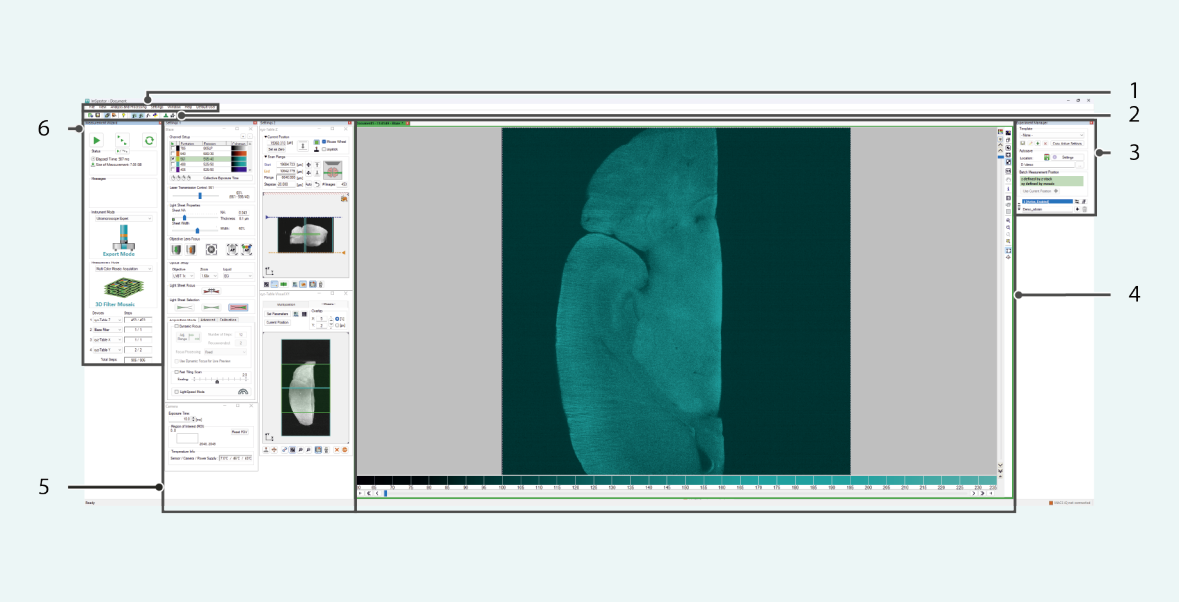


Figure 4.1: Graphical user interface

1	Menu bar	4	Stack window
2	Toolbar	5	Settings pane
3	Experiment Manager	6	Measurement Wizard

4.1 Menu bar

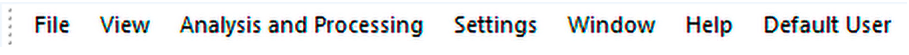


Figure 4.2: Menu bar

The menu bar provides access to the following menus:

- File
- View
- Analysis and Processing
- Settings
- Window
- Help
- Default User

4.1.1 File menu

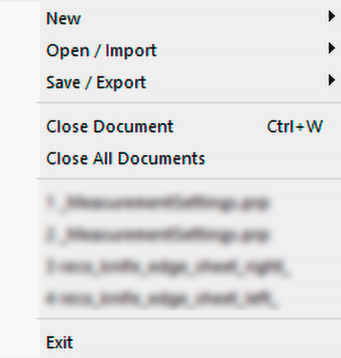


Figure 4.3: File menu

New	Creates a new document, a new stack window, or a graph window for the current document
Open / Import	Opens or imports documents or templates
Save / Export	Saves or exports documents or templates
Close Document	Closes the currently opened document window
Close All Documents	Closes all document windows
Exit	Closes the software

Table 4.1: File menu

All other previously listed opened files are also shown in this menu.

4.1.2 View menu

All dialogs are listed in the **View** menu.

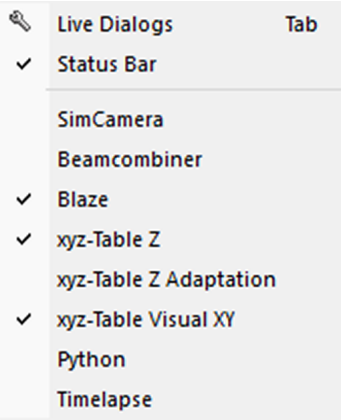
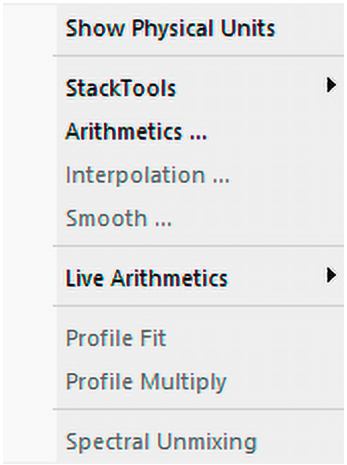


Figure 4.4: View menu

Live Dialogs	Shows all live dialogs
Status Bar	Shows or hides the status bar

Table 4.2: View menu

4.1.3 **Analysis and Processing** menu



Show Physical Units	Shows physical units as unit of length
StackTools	Selects the stack tools
Arithmetics	Opens the **Arithmetic Operations** dialog
Live Arithmetics	Selects the arithmetic tools
Profile Fit	*Not used for this instrument*
Profile Multiply	*Not used for this instrument*
Spectral Unmixing	*Not used for this instrument*

Table 4.3: Analysis and Processing menu

4.1.4 Settings menu

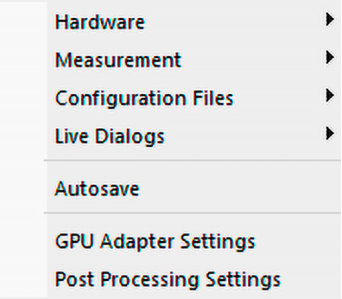


Figure 4.5: Settings menu

Hardware	Opens a menu to change the hardware configuration; some changes may require support by Miltenyi Biotec
Measurement	Opens a menu to change advanced measurement configuration settings and show or hide buttons or boxes
Configuration Files	Opens a menu to change configuration files; some changes may require support by Miltenyi Biotec
Live Dialogs	Changes the settings for visibility and position of dialogs
Autosave Settings	Changes the settings for autosave, for example, the storage location
User Settings	Changes the user settings
Remote Control Settings	Changes the remote control settings *(only used if two microscopes need to be synchronized)*
GPU Adapter Settings	*Not used for this instrument*
Post Processing Settings	*Not used for this instrument*

Table 4.4: Settings menu

4.1.5 Help menu

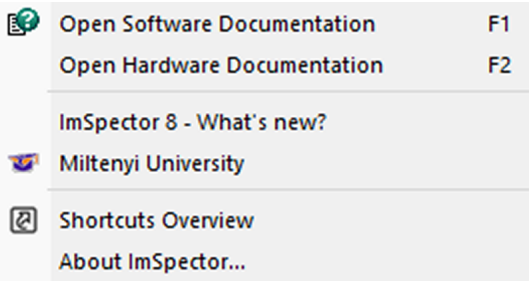


Figure 4.6: Help menu

Open Software Documentation	Opens the software documentation as an HTML file
Open Hardware Documentation	Opens the hardware documentation as a PDF file
What's new?	Opens the Release Notes
Miltenyi University	Opens the learning platform Miltenyi University in a web browser
Shortcuts Overview	Opens a list of keyboard shortcuts
About ImSpector	Shows information about the software

Table 4.5: Help menu

4.1.6 User menu

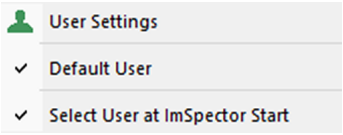


Figure 4.7: User menu

User Settings	Opens a dialog to set up user profiles
Default User	Selects the default user or changes the active user
Select User at ImSpector Start	Activates selecting the active user when opening the software

Table 4.6: User menu

4.2 Toolbar

The toolbar provides quick access to often used functions and features.

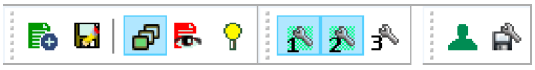


Figure 4.8: Toolbar

New Document	Creates a new document
Image Series Viewer	Shows or hides the **Image Series Viewer**
Save as	Saves a document
Dark Mode	Turns on the **Dark Mode**, which softens the screen with a dark theme
Settings 1	Shows or hides the Settings 1 pane; currently shown

	**Settings 2**	Shows or hides the Settings 2 pane; currently shown
	**Settings 3**	Shows or hides the Settings 3 pane; currently hidden
	**Python**	Opens the Python script editor
	**User Settings**	Opens the **User Settings** dialog
	**Save Settings**	Saves the settings as default

Table 4.7: Buttons of the toolbar

5 Live Dialogs

This chapter describes the **Live Dialogs** used to configure and control various components of the instrument. Each component has its own dialog that asks for input.

5.1 Rearranging the dialogs

You can arrange the individual dialogs in up to three settings panes on the left-hand side of the stack window.

1 To show or hide each pane, click **Settings > Live Dialogs > Show / Hide**.

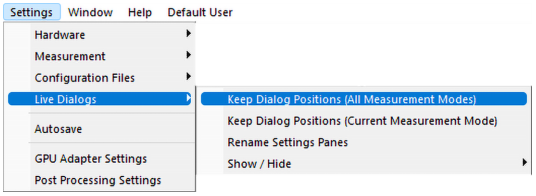
2 To dock and undock panes, double-click the corresponding title bar.

3 To show or hide all panes except the **Measurement Wizard**, press the Tab key.

The availability of the dialogs depends on the **Instrument Mode** and **Measurement Mode**.

1 Select the dialog layout you want to use:

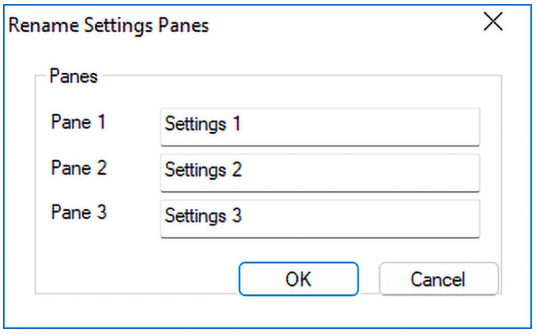
- To save the dialog layout for the current **Instrument Mode** and for each **Measurement Mode**, select **Settings > Live Dialogs > Keep Dialog Positions (All Measurement Modes)**.



or

- To save the dialog layout for the current **Instrument Mode** and for the current **Measurement Mode**, select **Settings > Live Dialogs > Keep Dialog Positions (Current Measurement Mode)**.

2 Optional: To rename each pane, select **Settings > Live Dialogs > Rename Settings Panes** and enter the new name.



3 To show or hide each pane, select **Settings > Live Dialogs > Show / Hide**.

5.2 Using the dialogs

You can use the dialogs to control various aspects of the instrument. For use cases, refer to the instrument user manual.

5.2.1 Channel Setup

In the **Channel Setup**, you can find all available excitation filters and emission filters for the instrument. The list shows a checkbox followed by **Excitation** wavelength, **Emission** wavelength, and a configurable **Colormap**.

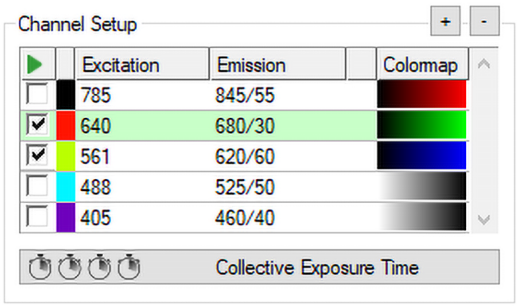
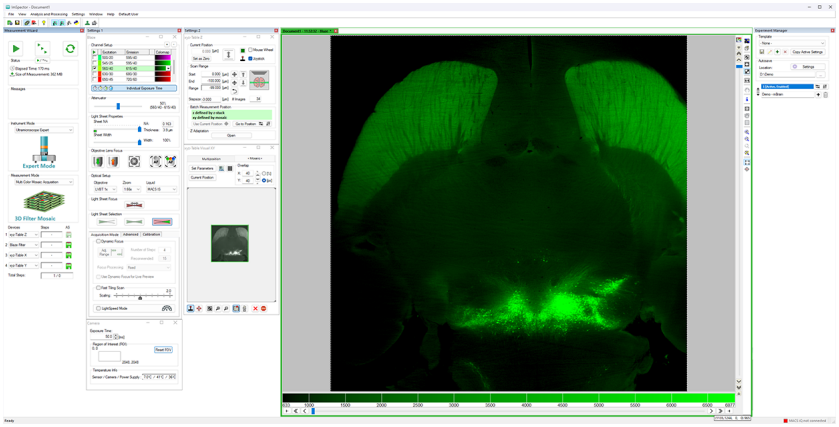


Figure 5.1: Channel Setup

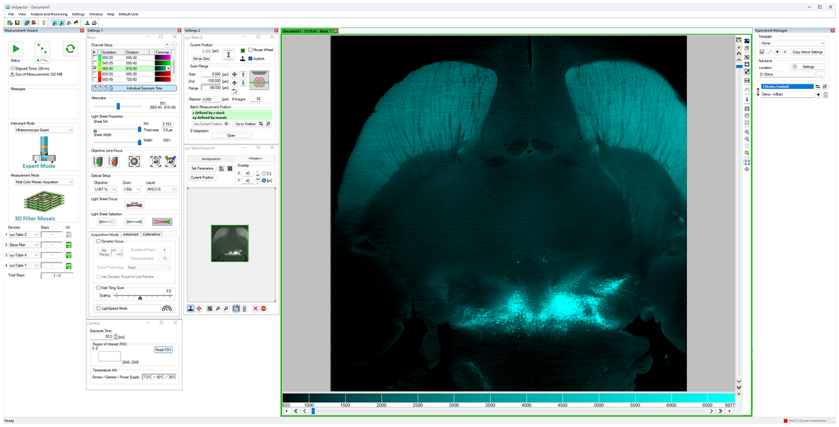
To change the colormap:

1 Click the colormap for the respective excitation wavelength and emission wavelength.



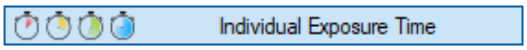
2 Select the desired color.

The stack is automatically adjusted.



To set individual exposure times for the selected channels:

1 Click the **Collective Exposure Time** button. The button changes to **Individual Exposure Time**.



To set collective exposure times for the selected channels:

1 Click the **Individual Exposure Time** button. The button changes to **Collective Exposure Time**.



5.2.2 Light Sheet Properties

To change the numerical aperture (NA) of the light sheet and its width, you can use **Light Sheet Properties**.

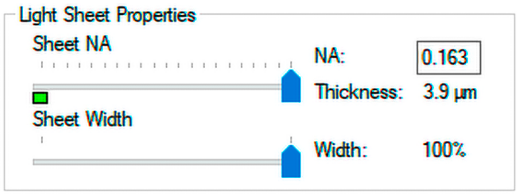


Figure 5.2: Light Sheet Properties

1 Move the slider under **Sheet NA** to adjust the thickness of the light sheet. A higher NA creates a thinner light sheet near the light sheet focus. A lower NA creates a more homogeneous light sheet, but reduces the resolution in the z-plane.

The shown thickness of the light sheet is only a reference value. The quality of the sample and the alignment of the instrument can also have an effect on the thickness of the light sheet.

2 Move the slider under **Sheet Width** to adjust sheet width of the light sheet. A lower sheet width results in more light being focused on a smaller region of the sample.

If the sheet width is reduced too much, graduated or incomplete illumination of the field of view may occur.

5.2.3 Objective Lens Focus

Move Detection Unit Down	Moves the detection unit to the measurement position; must be held clicked for safety reasons.
Move Detection Unit Up	Moves the detection unit to the park position.
Manual Focus	Activates the slider to focus manually.
Single Channel Autofocus	Focuses automatically on current channel. When clicked, the detection unit goes through a short path in z-direction. The software selects the z-position with the strongest contrast. Right-click causes to toggle autofocus on the ROI.
Multichannel Autofocus	Focuses automatically on all selected channels in the **Channel Setup**. Right-click causes to focus only on the ROI.

Table 5.1: Buttons of the **Objective Lens Focus** dialog

Autofocus is applied to the reference channel first, as it affects the focus position of all other channels. When focusing manually, the reference channel must be focused first.

5.2.4 Optical Setup

To select the correct settings for **Objective**, **Zoom**, and **Liquid** of the experiment, you can use **Optical Setup**.

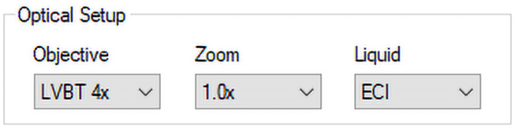


Figure 5.3: Optical Setup

The selected **Liquid** must match the liquid in the cuvette because the light sheet focus depends on the refractive index of the liquid used. Additional liquids can be added to the preset ones.

5.2.5 Light Sheet Focus

To toggle the visibility of the light sheet focus indicator in the stack window, you can click the **Show Light Sheet focus adjustment** button under **Light Sheet Focus**.

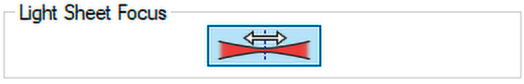


Figure 5.4: Light Sheet Focus

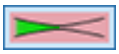
5.2.6 Light Sheet Selection

To select the optimal light sheet setting for the experiment, you can use **Light Sheet Selection**.



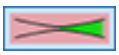
Figure 5.5: Light Sheet Selection

To select only the left light sheet:



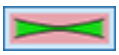
1 Click **Select left light sheet**.

To select only the right light sheet:



1 Click **Select right light sheet**.

To select both light sheets:



1 Click **Select both light sheets**.

5.2.7 **Calibration** tab for excitation

To set a reference point for the software focus position to the actual position of the light sheet focus, you can use the **Calibration** tab. Use the marker at the top of the slider to set the global offset for the reference wavelength. You can fine-tune the chromatic correction for all other excitation wavelengths with the marker at the bottom. You can use the **Set as Reference** button to set the current excitation wavelength as the new reference wavelength. If the **Coupled Chromatic Correction** checkbox is selected, the chromatic correction is applied symmetrically to the left and right light sheet. Otherwise, the chromatic correction can be set individually for each light sheet.

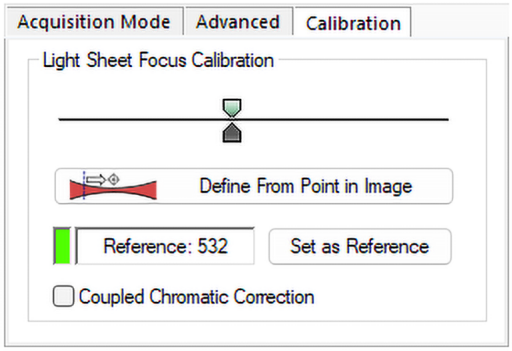


Figure 5.6: Calibration tab

5.2.8 **Advanced** tab

To change the **Light Sheet Processing** and the **Light Sheet Configuration**, you can use the **Advanced** tab.

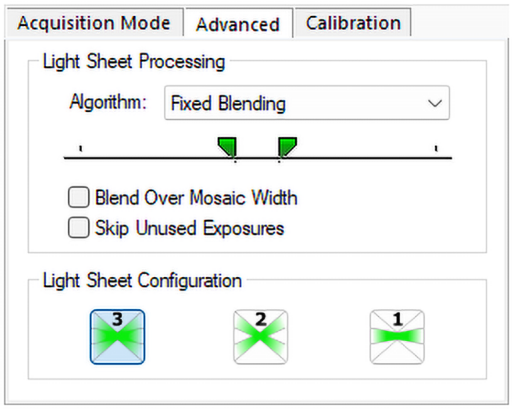
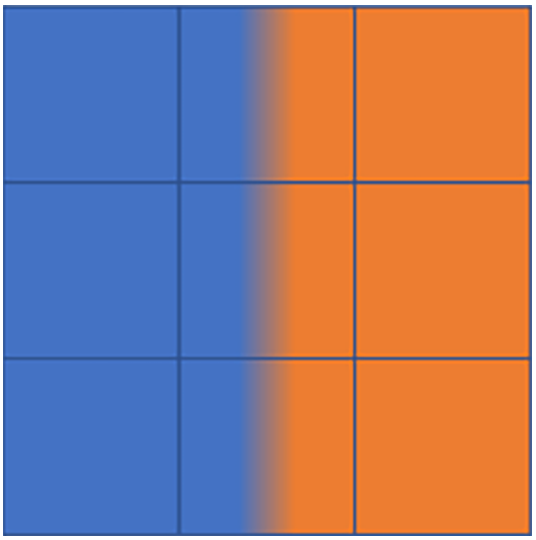


Figure 5.7: Advanced tab

To use the image algorithm **Fixed Blending**:

1 Select **Skip Unused Exposures**. By this, all exposures that are not going to contribute to the processed image are skipped.

2 Select **Blend Over Mosaic Width** to blend over the whole mosaic width. The following example shows a 3 × 3 mosaic, in which the left-hand side is not covered by the right light sheet (orange) and the right-hand side is not covered by the left light sheet (blue). For the middle, both light sheets are cross-faded.



The **Blend Over Mosaic Width** combo box is not available if either the left or right light sheet is selected or if the x-axis is not one of the active devices.

If **Blend Over Mosaic Width** is selected, the acquisition speed can be increased and a lower value under **Laser Transmission Control** can be used to decrease photobleaching.

If you activate **Dynamic Focus**, the number of focus steps is limited to the ROI areas that remain visible after both image sides are blended.

To compensate errors from a poor sheet focus calibration for the **Light Sheet Processing**:

1 Select **Adaptive Blending** under **Algorithm**.

Under **Light Sheet Configuration**, you can select which laser beams are active.

5.2.9 Acquisition Mode tab

In the **Acquisition Mode** tab, you have two options:

- Dynamic Focus
- Fast Tiling Scan

Depending on your ordered configuration, **LightSpeed Mode** as a third option might be visible.

Dynamic Focus

To activate the dynamic focus in the **Acquisition Mode** tab, you have to select the **Dynamic Focus** checkbox.

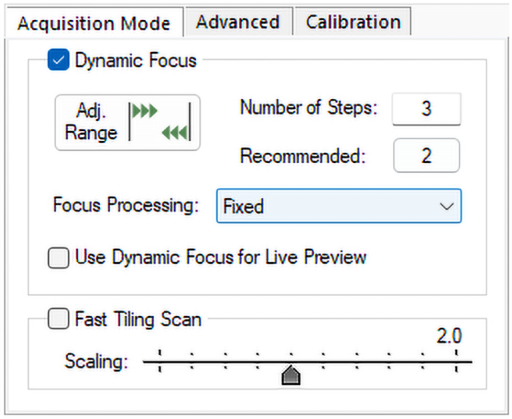


Figure 5.8: Dynamic Focus mode

With dynamic focus enabled, the camera exposure time is matched to the focus travel time for synchronized image acquisition.



1 To show or hide the horizontal focus range indicators and adjust the focus range in the stack window, click the **Show dynamic focus range adjustment** button. During a measurement with enabled dynamic focus, only the area between the indicators will be covered by the light sheet focus.

2 Adjust the range of the dynamic focus to cover the region of interest.

3 Enter the **Number of Steps** or click **Recommended** to select the number of steps taken.

Consider that the **Number of Steps** corresponds to the number of recorded raw images. A large number of steps increases the image acquisition time and the amount of data significantly. The recommended number of steps approximates the number of images necessary to achieve a homogenous z-resolution.

To blend between the theoretical focus positions:

1 Select **Fixed** under **Focus Processing**. The results are best with proper sheet focus calibration in poor samples.

To estimate the sheet focus position during the measurement:

1 Select **Adaptive** under **Focus Processing**. This setting can compensate for bad sheet focus calibration.

To use the dynamic focus during live preview:

1 Select the **Use Dynamic Focus for Live Preview** checkbox.

With dynamic focus activated during live preview, the acquisition time is longer.

5.2.10 **xyz-Table Z** view

To move the sample in z-direction, you can use the **xyz-Table Z** view. The sample can be moved by the jog wheel or the mouse wheel. For the use of the mouse wheel, the cursor has to be kept on the brain icon.

This view has the following group boxes, which can both be collapsed:

- **Current Position**
- **Scan Range**

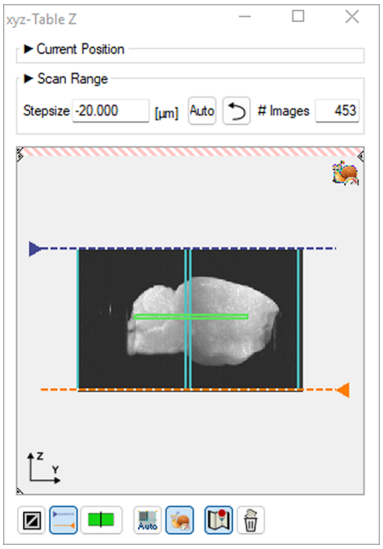


Figure 5.9: xyz-Table Z view

The following table describes the buttons, which are only available in this view.

Show/Hide start and end points	Shows/Hides start and end point of the z-range
Toggle to show a MIP for the left, right or both light sheet sides fused together	Toggles showing a MIP for the left side, right side, or both sides of the light sheet that have been fused together (both light sheet sides is shown)
Apply speed limit of z-movement during live preview to avoid motion blur in MIP (F3)	Slows down z-movement during live preview (“snail mode”) to avoid motion blur in MIP (F3)

Table 5.2: Buttons of the **xyz-Table Z** view

Current Position

After switching on the instrument, the xyz-Table moves to its start position. By entering the **Current Position**, you can change the start position in z-direction. By clicking **Set as Zero**, you can set a new reference point of the sample. By selecting **Mouse Wheel** or **Joystick**, you can set the input device.



Figure 5.10: Current Position

Scan Range

Within this group box, you can configure the scan range together with the step size and the number of images. The start and end values can be typed into the fields, as well as the step size or the number of images. The range is automatically calculated. Clicking the **Set current position as start** button and the **Set current position as end** button applies the position set in the **Current Position** group box. The step size can automatically be set to 50% of the light sheet thickness by clicking the **Auto** button, effectively linking it to the **Sheet NA** definition.

Alternatively, you can move the xyz-Table to the start position and the end position by clicking the corresponding arrow. By entering values for **Step Size** or **# Images**, you can define the number of images taken.

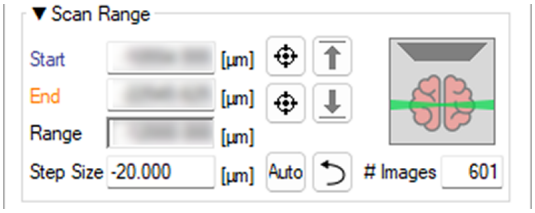


Figure 5.11: Scan Range

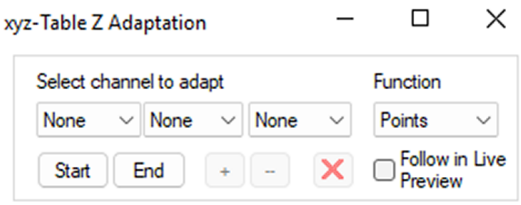
Set current position as start/end	Sets the current position as start/end point
Move to start position	Moves the sample to the desired start position
Move to end position	Moves the sample to the desired end position
Set the step size automatically	Toggles setting the optimal step size automatically (default is 0)
Reset dialog to its original state	Resets the values to its default

Table 5.3: Buttons of the **Scan Range** group box

5.2.11 Z Adaptation

To adjust the laser power relative to the z-position:

1 Open the **xyz-Table Z Adaptation** view.



2 Define a z-stack (see **Acquiring an image on page 35**).

3 Select the channel that shall be used.

4 Move to the start position.



5 Define the laser power at the start position and click **Start**.

6 Move to the end position.



7 Define the laser power at the end position and click **End**.

8 Select the **Function** of choice: **Points** or **Linear** are recommended.

9 Optional: To adapt the laser power during live preview, select the **Follow in Live Preview** checkbox. By this, the detection unit and/or sample can be moved in z, and the laser power is adapted in real time.

5.2.12 **xyz-Table Visual XY** view

To move the sample in xy-direction, you can use the **xyz-Table Visual XY** view. The sample can be moved by the jog wheel. The field of view is represented by the green tile.

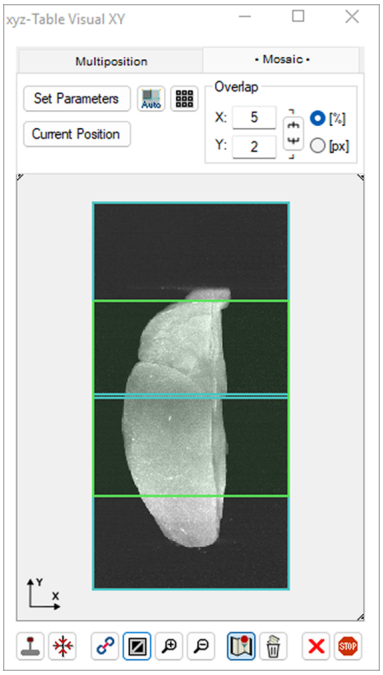


Figure 5.12: Mosaic tab

This view has two tabs: one for **Mosaic** and one for **Multiposition** measurements. The following table describes the buttons, which are available in both tabs.

Allow the joystick to move to the current position	Activates or deactivates the jog wheel to move to the current position
Set current position as origin	Sets the current position as origin
Link XY and Z dialog. Hides the buttons in the Z dialog	Activates or deactivates linking the **xyz-Table Visual XY** dialog and the **xyz-Table Z** dialog (should be activated by default)
Reset view to display all positions	Resets view to display all positions
Delete all positions of this mode	Deletes all positions of this measurement mode
Stop	Stops the xy-movement immediately and stops the measurement
Zoom in	Zooms into the view
Zoom out	Zooms out of the view
Capture overview MIP at current position	Acquires overview maximum intensity projection (MIP) at current position (see **Simple view on page 51**)
Clear overview MIP	Clears overview MIP
Define mosaic automatically	Defines mosaic automatically when overview MIP is used (only available in the **Mosaic** tab)

Convert Mosaic mode positions to Multiposition mode	Converts **Mosaic** mode positions to **Multiposition** mode (only available in the **Mosaic** tab)
Set overlap in x and y to the same value (OFF state)	Activates or deactivates the same overlap value in x and y (in percent or pixel) (only available in the **Mosaic** tab)
Show order of measurement positions	Shows the ascending order of the measurement positions (only available in the **Multiposition** tab)
Define order of measurement positions	Defines the descending order of measurements to reduce the movement of the sample (only available in the **Multiposition** tab)
Set z-offsets for individual positions	Activates or deactivates z-offsets for different positions (only available in the **Multiposition** tab)

Table 5.4: Buttons of the **xyz-Table Visual XY** view

Mosaic measurements

Observe the instructions for creating a mosaic measurement under **Acquiring a 3D mosaic image on page 37** or **Acquiring a multicolor mosaic image on page 38**.

To make more precise changes to the experiment, you can click the **Set Parameters** button. By this, the **Sizes of One Image** and the **Number of Images** can be set.

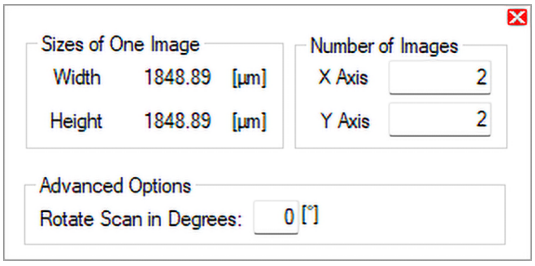


Figure 5.13: Set Parameters dialog

Rotate Scan in Degrees must be set to zero.

To edit the current position in x- and y-direction, you can click the **Current Position** button and enter numerical values.

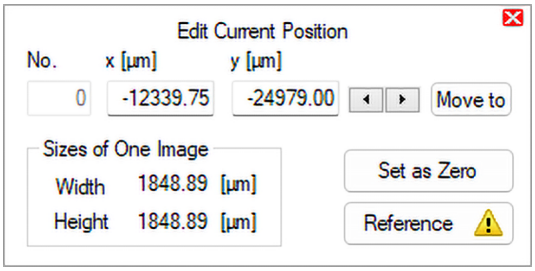


Figure 5.14: Current Position dialog

Multiposition measurements

Observe the instructions for creating a multiposition measurement under **Acquiring a 3D multiposition dataset on page 39**.

To retrieve more details on the individual positions, click the **Edit Position** button. You can move through all active positions by using the arrows or the **Move to** button. Alternatively, you can enter numerical values for the current position. Select an active position to show its position and highlight it in dark gray color.

To set the coordinates of one position as reference point, you can click **Set as Zero**. Clicking **Reference** moves the xyz-Table to this reference point.

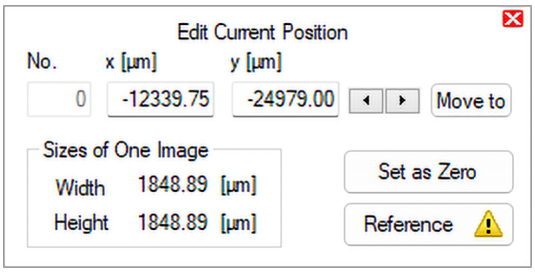


Figure 5.15: Current Position dialog

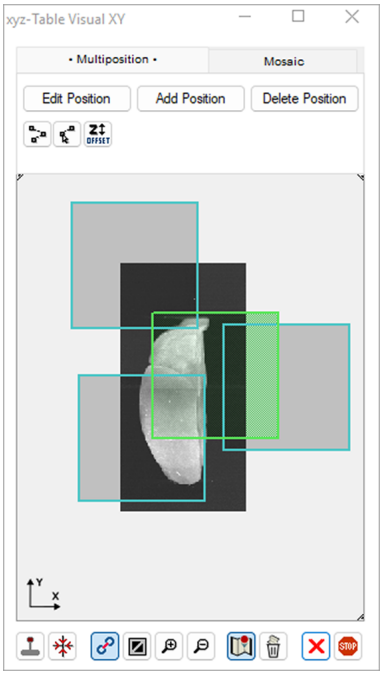


Figure 5.16: Multiposition tab

To show the ascending order of the measuring positions, you can click the **Show order of measurement positions** button.

To set the ascending order of measuring positions, you can click the **Define order of measurement positions** button.

To activate setting different z-offsets when clicking the **Edit Position** button, you can click the **Set Z offsets for individual positions** button.

5.2.13 Camera

To control the camera settings of the instrument, you can use the **Camera** view. **Temperature Info** shows the temperature of the sensor, the camera, and the power supply.

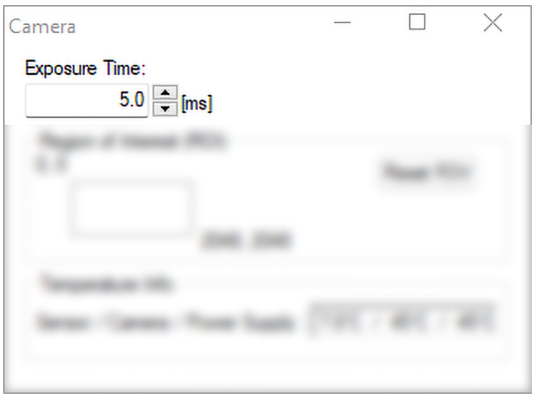


Figure 5.17: Camera view

1 Set an **Exposure Time** depending on the sample used. A suitable starting value can be anything between 100 and 200 ms.

6 Setting up an experiment

This chapter describes how to set up basic experiments. For more detailed information on the operation of the instrument, including light sheet alignment, refer to the instrument user manual.

6.1 Measurement Wizard

With the **Measurement Wizard**, you can start and stop a measurement or start a live preview in the document window. If activated under **Settings > Measurement**, you can also start a batch measurement.

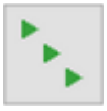
 **Start Measurement**	Starts the defined measurement
 **Start Measurement**	Starts the defined measurement in LightSpeed Mode
 **Stop Measurement**	Stops the defined measurement
 **Start Batch Measurement**	Starts the defined batch measurement
 **Start Live Preview**	Starts a live preview
 **Toggle Estimation**	Toggles the estimation of parameters for the active measurement

Table 6.1: Buttons of the **Measurement Wizard**

After setting up an experiment, the amount of necessary storage space is shown under **Status** as well as the estimated time. Under **Messages**, information and warnings for the measurement setup are shown.

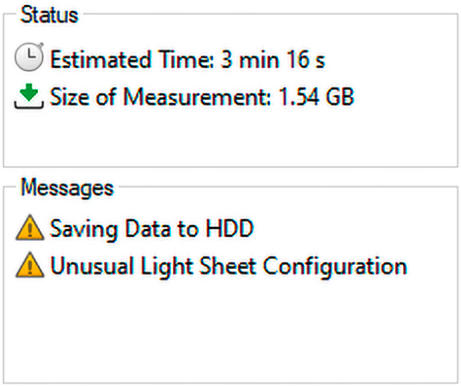


Figure 6.1: Status and **Messages** area

6.2 Instrument Mode

In the **Measurement Wizard**, you can select the **Instrument Mode** for the ordered configuration.

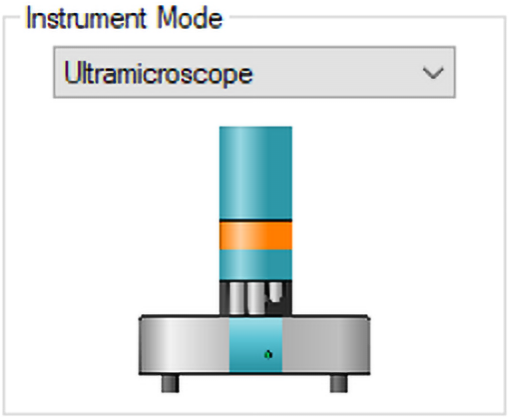


Figure 6.2: Instrument Mode list

All instrument modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See **Technical support on page 65**.

6.3 Measurement Mode

To acquire data, you must define the order of the devices to be used during acquisition. You can freely define these devices by selecting them in the **Devices** lists or use predefined settings, called measurement modes. The available measurement modes can be selected in the **Measurement Mode** list.

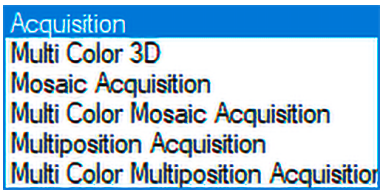


Figure 6.3: Measurement Mode list

All measurement modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See **Technical support on page 65**.

Deactivating the **Autosave** function for the **xyz-Table Z** is recommended.

6.3.1 Acquiring an image



1 To start the live preview, click the **Start Live Preview** button in the **Measurement Wizard**.

2 Move in the z-direction to the start position.

3 Click **Current Position** > **Set as Zero** in the **Settings 2** pane.



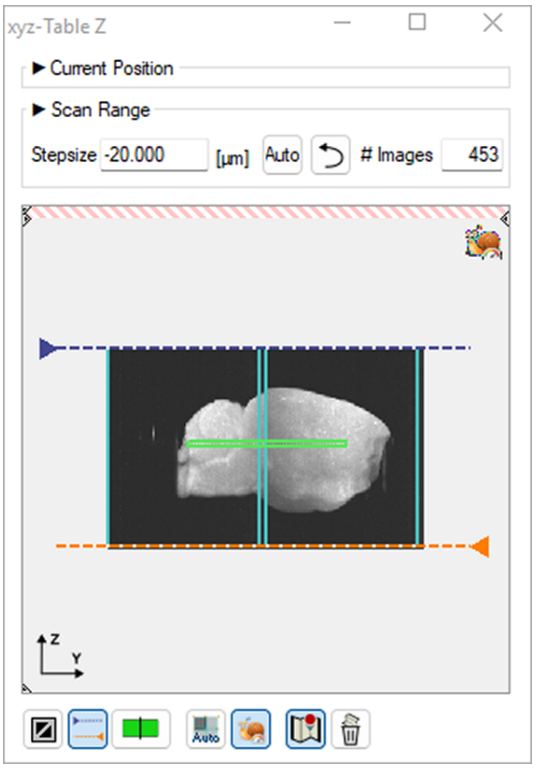
4 Click the **Set current position as start** button under **Scan Range** > **Start**.

5 Move in the z-direction to the end position.



6 Click the **Set current position as end** button under **Scan Range** > **End**.

7 Define the step size, corresponding to the number of images, by entering it under **Scan Range** > **Stepsize**. Alternatively, click the **Auto** button.





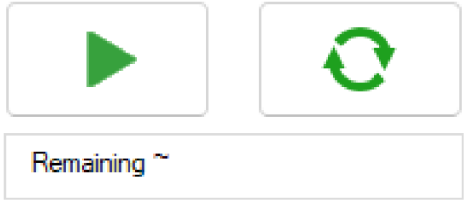
8 To stop the live preview, click the **Stop Live Preview** button. The laser is switched off.

9 Check **Settings** in the **Autosave** group box of the **Experiment Manager**.



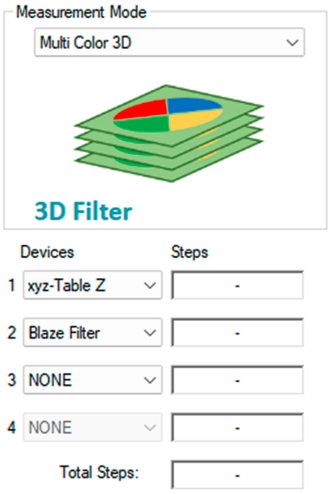
10 **CAUTION! Crushing of hands hazard. Keep a safe distance from the instrument while the acquisition is in progress.** To start the acquisition, click the **Start Measurement** button.

The xyz-Table performs a reference run.

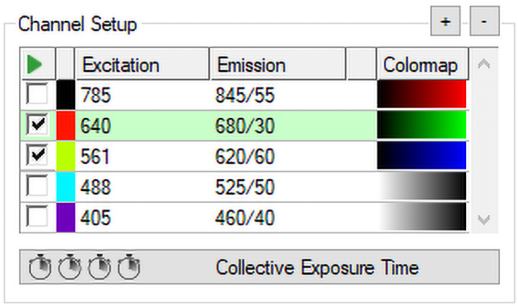


6.3.2 Acquiring a multicolor z-stack

1 In the **Measurement Wizard**, select **Measurement Mode > Multi Color 3D**.



2 Select the correct excitation and emission wavelengths for your sample under **Channel Setup** in the **Settings 1** pane. The selected checkboxes are used.



3 **NOTICE Higher laser power bleaches the sample faster.** Move the slider under **Laser Transmission Control** while the filter is highlighted to set the laser power for each individual filter.

4 Select the shortest emission wavelength, and create a sharp picture.



5 Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction**, starting with the next longer wavelength.



6 If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.

7 To start the acquisition, proceed as described under **Acquiring an image on the previous page**.

6.3.3 Acquiring a 3D mosaic image

1 In the **Measurement Wizard**, select **Measurement Mode > Mosaic Acquisition**.



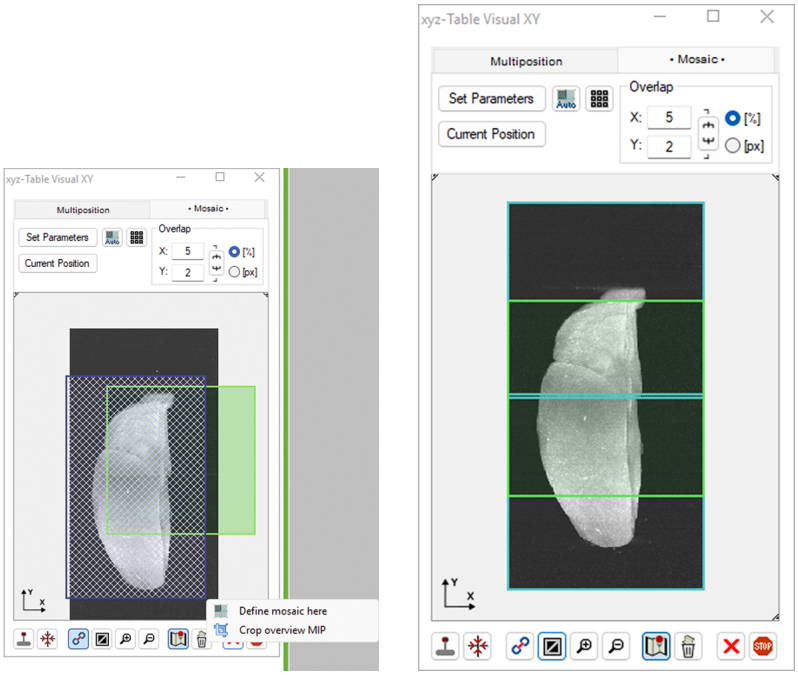
2 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.

3 Delete all positions of this **Measurement Mode**.

4 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).

The overlap must be at least 40 px.

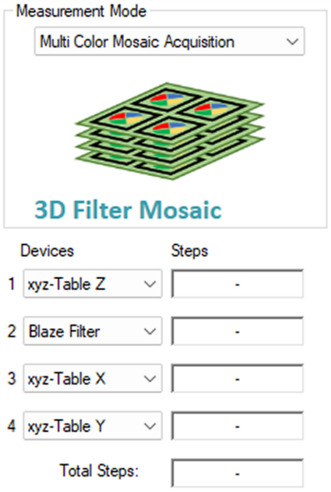
5 To draw a ROI you want to cover with a mosaic, click-drag with the right mouse button. From the pop-up menu, select **Define mosaic here**.



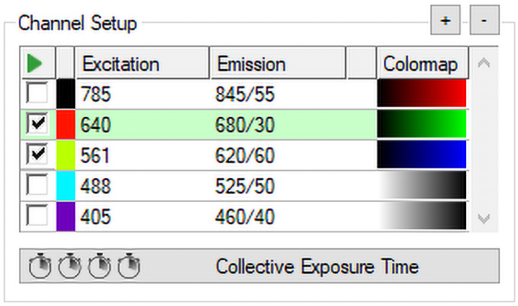
6 To start the acquisition, proceed as described under **Acquiring an image on page 35**.

6.3.4 Acquiring a multicolor mosaic image

1 In the **Measurement Wizard**, select **Measurement Mode > Multi Color Mosaic Acquisition**.



2 Select the correct excitation and emission wavelengths for your sample under **Channel Setup** in the **Settings 1** pane. The selected checkboxes are used.



3 **NOTICE Higher laser power bleaches the sample faster.** Move the slider under **Laser Transmission Control** while the filter is highlighted to set the laser power for each individual filter.

4 Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction**, starting with the next longer wavelength.

5 If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.

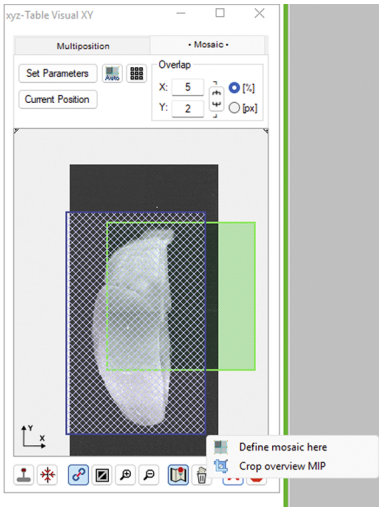
6 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.

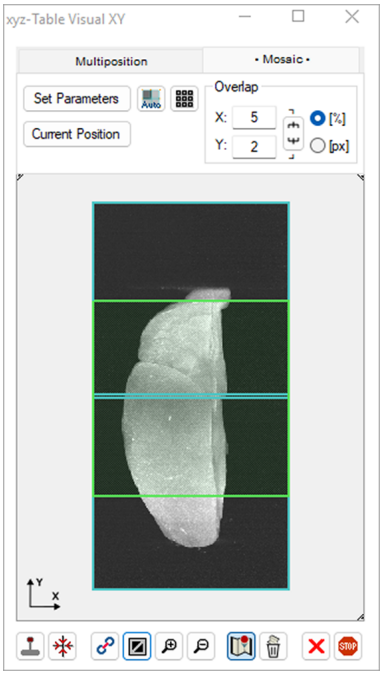
7 Delete all positions of this **Measurement Mode**.

8 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).

The overlap must be at least 40 px.

9 To draw a ROI you would like to cover with a mosaic, click-drag with the right mouse button. From the pop-up menu, select **Define mosaic here**.





10 To start the acquisition, proceed as described under **Acquiring an image on page 35**.

6.3.5 Acquiring a 3D multiposition dataset

1 In the **Measurement Wizard**, select **Measurement Mode > Multiposition Acquisition**.

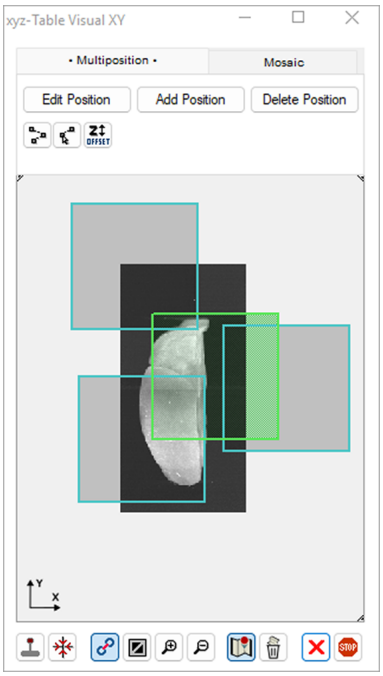


2 In the **xyz-Table Visual XY** view, click the **Multiposition** tab.

3 Delete all positions of this measurement mode.

4 Move the measurement positions to your region of interest.

5 Click **Add Position** for each position you want to add.



6 To start the acquisition, proceed as described under **Acquiring an image on page 35**.

6.4 Experiment Manager

The **Experiment Manager** is located by default on the far right of the ImSpector software. If not, deactivate hiding it under **Window > Hide Experiment Manager**. For the function of the individual buttons, see **Table 6.2**.

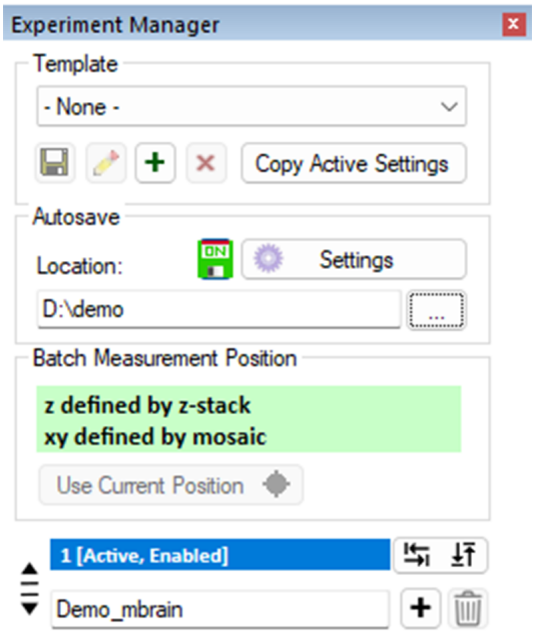


Figure 6.4: Experiment Manager

With the **Experiment Manager**, you can save, load, or configure predefined templates and set up experiments. Default templates are provided by Miltenyi Biotec. These are specialized templates for the use in combination with the multisample holders. If a template is changed and not saved, ImSpector asks if the settings shall be updated for the current template before closing.

Within the **Batch Measurement Position** group box, you can store the current xyz-Table position as the measurement reference position (x/y/z) by clicking the **Use current position** button for the active measurement. If xy is defined by a mosaic or a multiposition acquisition, those definitions take precedence.

In batch measurements, which consist of many single measurements, different statuses are possible. **Active** means that this measurement has focus. **Enabled** means that this measurement will be performed if a batch measurement is started. The statuses are indicated by a color code. See **Table 6.3**.

You can copy settings from the active measurement by clicking **Copy Active Settings**. The following window is shown:

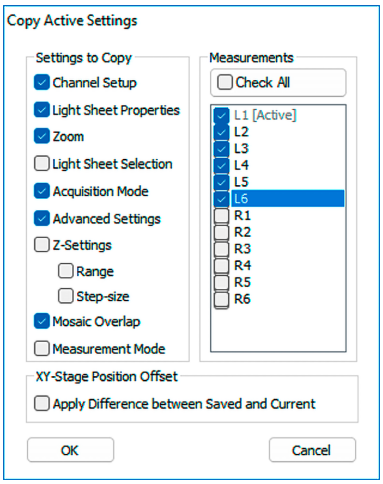


Figure 6.5: Copy Active Settings window

You can select the settings to be copied to other measurements on the left and the measurements to which those settings are applied on the right. To select all available measurements, you can use the **Check All** checkbox.

The **Settings** button in the **Autosave** group box provides more advanced options to modify your data output format. The **Autosave** button toggles autosave for all axes of the selected measurement.

	**Save current settings**	Saves an open template
	**Rename selected custom template**	Renames the selected custom template; default templates cannot be renamed
	**Add custom template with current settings**	Creates a new template from current settings
	**Delete selected custom template**	Deletes the selected template
	**Toggle autosave**	Activates or deactivates autosave for all axes of the selected measurement
	**Create new measurement**	Adds a further measurement to the experiment
	**Remove measurement**	Removes the selected measurement from the experiment
	**Activate and move stage to defined measurement position**	Activates the selected measurement and moves the xyz-Table (stage) to the defined position

Table 6.2: Buttons of the **Experiment Manager**

Color	Status
Dark blue	**Active, Enabled**
Light blue	**Inactive, Enabled**
Yellow	**Active, Disabled**
Grey	**Inactive, Disabled**
Red	**Active, Enabled** current xyz

Table 6.3: Color code of measurement status

6.5 Setting up a batch measurement

With the batch measurement function, you can perform multiple measurements sequentially without additional intervention. These measurements can be saved as a template.



1. In the **Experiment Manager**, click the **Create New Measurement** button.
2. Name your measurement by right-clicking the colored top bar. An autosave prefix has also to be set, for example, MyBrainSample.
3. Define the measurement settings for your measurement. See **Setting up an experiment on page 33**.
4. If required, click the **Create New Measurement** button repeatedly to add further measurements. By dragging the indicator on the left-hand side, the order is changed.
5. If required, click the **Use Current Position** button under **Batch Measurement Position**, or define a mosaic.



6. Click the **Go to Position** button to move the xyz-Table to the defined position.
7. Optional: By right-clicking, the selected measurement can be made inactive.



8 To start the acquisition, click the **Start Batch Measurement** button.

For safety reasons, it is impossible to change the objective lenses during a batch measurement.

7 Data visualization and analysis

This chapter describes the visualization and analysis of acquired data. The acquired data is shown in the stack window of the ImSpector software. Once the data is saved, you can open it via the **Image Series Viewer**. The data can be broken down into its constituent parts, examined, and analyzed.

7.1 Stack window

The stack window in the ImSpector software shows the acquired data:

- The file name is shown in the header.
- The toolbar located on the right of the image contains buttons for frequently used data handling functions. See **Table 7.1**.
- Some options can be accessed by right-clicking the image. See **Table 7.2**.

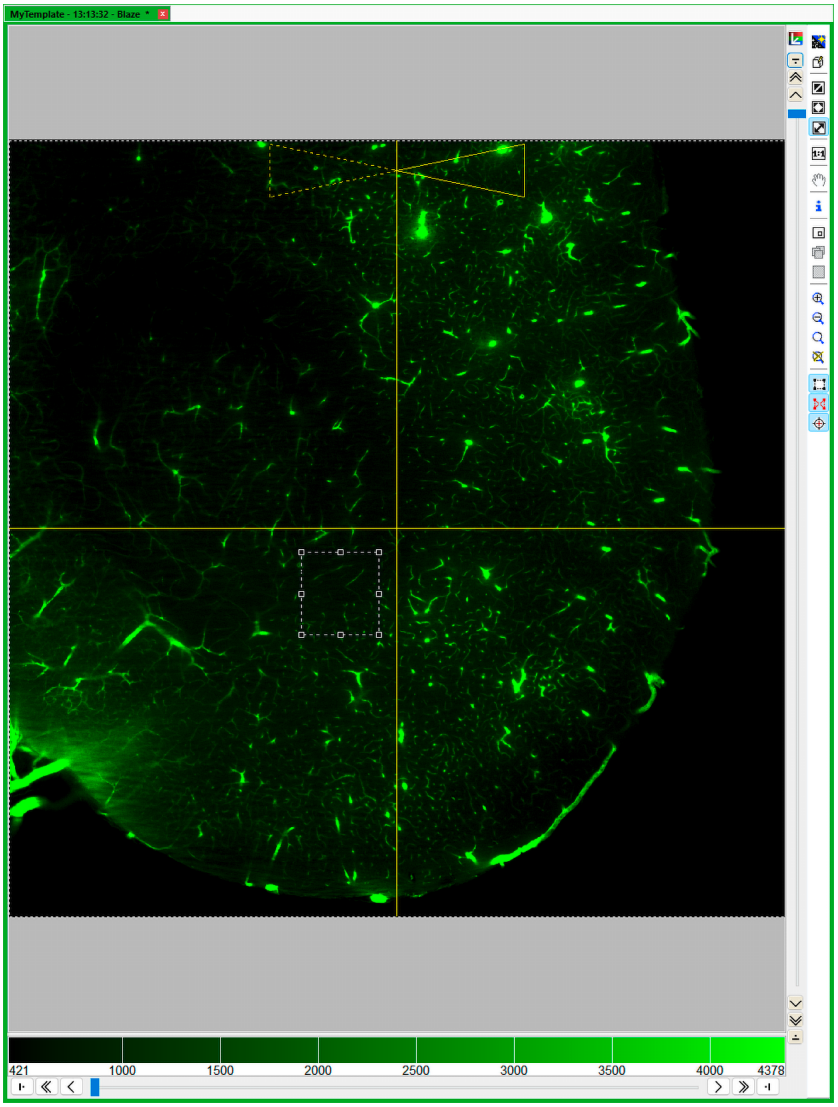


Figure 7.1: Stack window

The row below the image shows the colormap, which is used to display the different pixel values in different colors. You can get additional information for each pixel by moving the cursor over it.

Display Settings	Opens the **Display Settings** dialog below the stack window.
Adjust Window/Level	Adjusts the contrast of the colormap automatically to the minimum and maximum pixel value within the whole image or a shown region of interest (ROI).
Change Size and Dim	Opens the **Stack properties** window to enable a change in size and dimension of the data stack.
Fit Frame to Image	Fits the frame to the image. The aspect ratio is kept.
No Aspect Ratio	Fits the image to the frame. The aspect ratio is NOT kept.
Fit Image to Frame	Maximizes the image in a frame of constant size.
Show Image in Original Size	Shows the image in its original size.

	**Move the Image**	Grabs the image and moves it inside the frame; can only be used if the image is bigger than the frame.
	**Show Measurement Information**	Opens an additional window with metadata for the image.
	**Set as New ROI**	After drawing a ROI, this can be set as new camera or scan ROI. If the ROI is not shown, the button changes to **Reset ROI to full FOV** (field of view).
	**Show Merged Channel**	Shows an additional merged channel.
	**Show Merged Channel Only**	Shows only the merged image.
	**Zoom In**	Makes the image appear nearer.
	**Zoom Out**	Makes the image appear farther away.
	**Zoom to Selection**	Zooms into the ROI.
	**Reset Zoom**	Resets the zoom settings.
	**ROI for Active Profile**	Shows or hides the ROI.
	**Show Crosshair**	Shows or hides the crosshair.

Table 7.1: Buttons of the toolbar in the stack window

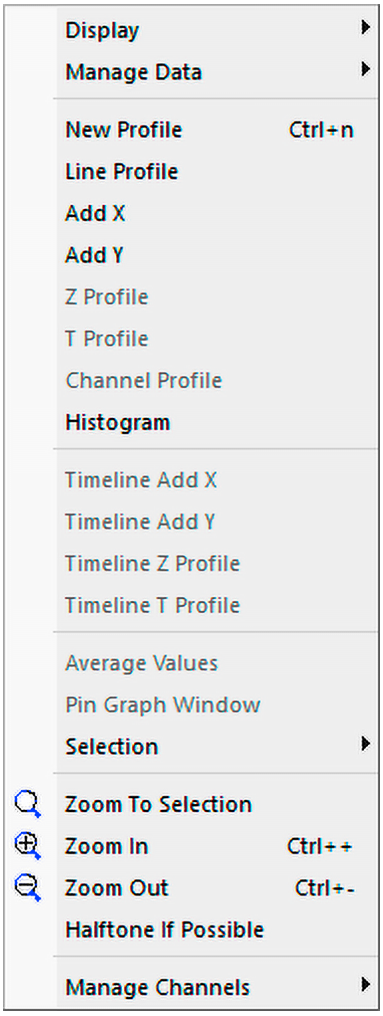


Figure 7.2: Right-click image options

Name	Function
Display	Changes display options
Manage Data	Copies, pastes, or edits data stacks
New Profile	Adds a new profile
Line Profile	Shows an intensity profile along a drawn line
Add X	Sums up all line functions in an interval x if a ROI was set
Add Y	Sums up all line functions in an interval y if a ROI was set
Z Profile	Shows an intensity profile around the z-axis (third data axis) if a ROI was set
T Profile	Shows an intensity profile around the t-axis (fourth data axis) if a ROI was set
Channel Profile	Selects the channel profile
Histogram	Shows a histogram if a ROI was set; the x-axis represents the pixel value and the y-axis how often the pixel values are present
Average Values	Shows the average values using **Add X** or **Add Y**
Group Plot Window	Pins a graph window to the stack window

Name	Function
Selection	Allows to change various ROI values
Halftone If Possible	Interpolates between pixels
Manage Channels	Allows to adjust various settings of the different color channels

Table 7.2: Right-click image options

7.2 Image Series Viewer

The Image Series Viewer lets you quickly review data that was saved directly to the hard drive via the **Autosave** function. The Image Series Viewer consists of three separate windows:

- **Image Series Browser**
- **Image Series Axes**
- **Image Series Manager**

7.2.1 Image Series Browser

The **Image Series Browser** provides an overview of all recent measurements of the current user.

	Measurement was completed
	Measurement was canceled
	Measurement data was successfully imported

Table 7.3: Status of measurement

7.2.2 Image Series Manager

With the **Image Series Manager**, you can import and export data, run a processing workflow, or reuse measurement settings.

7.2.3 Image Series Axes

In the **Image Series Axes** window, you can view a slice of a multidimensional data stack. In addition, you can define operations and destinations for individual axes. The selected operations will be applied when you import or export the data. Some operations, for example **Maximum intensity projection**, can be restricted to a range on the axis. The range is specified by additional indicators on the slider.

7.3 Stack Properties

In the **Stack Properties** dialog, you can see information about the stack itself.

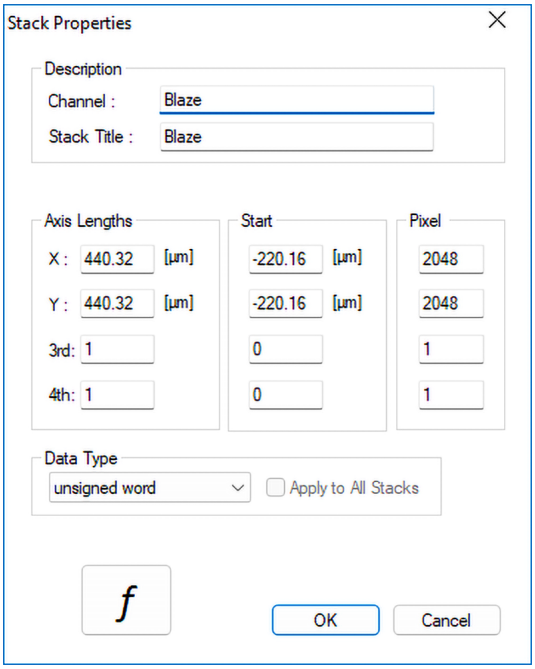


Figure 7.3: Stack Properties dialog

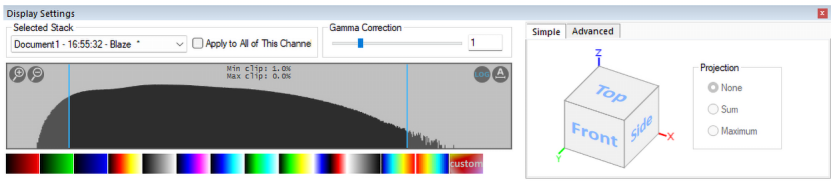
- The **Channel** and the **Stack Title** are shown in the **Description** box.
- The dimensions of the stack in µm are shown in the **Axis Lengths** box.
- The starting values in µm are shown in the **Start** box.
- The number of pixels and slices in all dimensions are shown in the **Pixel** box.
- The data type can be selected in the **Data Type** menu.
- The ***f*** button can be used to open the last used analysis tool again.

7.4 Changing the view

The ImSpector software can handle up to four-dimensional stacks. You can adjust the way acquired data is presented via the **Display Settings** dialog:



1 Click the **Display Settings** button to open the **Display Settings** dialog below the stack window.



The **Display Settings** dialog consists of two parts:

- Colormap dialog
- Axis handling dialog with **Simple** and **Advanced** settings

7.4.1 Colormap dialog

The ImSpector software uses colormaps to distinguish between different pixel values. The current range is shown below the image in the stack window. To change the corresponding settings, you can click a channel or a stack. Alternatively, you can select the channel or the stack directly under **Selected Stack**. If you select the **Apply to All of This Channel** checkbox, the colormap for all stacks is changed instead of only the selected one.

- Click the **Adjust Colormap Range** button to automatically adjust the colormap range.
- Move the blue bars with the mouse to adjust the colormap range manually.
- Move the slider under **Gamma Correction** to adjust the gamma correction.

The following table describes the other available buttons in this dialog.

Zoom In	Zooms into the selected histogram range
Zoom Reset	Resets the histogram zoom
LOG	Switches the scaling between linear and natural logarithmic
Adjust Colormap Range	Adjusts the colormap range automatically

Table 7.4: Buttons of the **Display Settings** dialog

7.4.2 Simple view

The **Simple** tab shows a cube with the sides **Top**, **Front**, and **Side**. Additionally, the axes x, y, and z are shown.

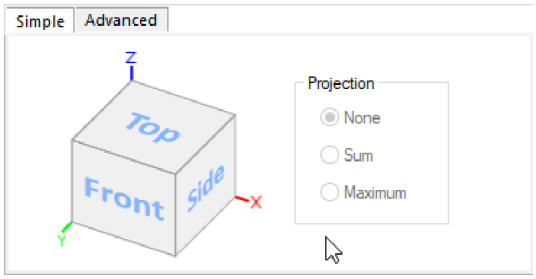
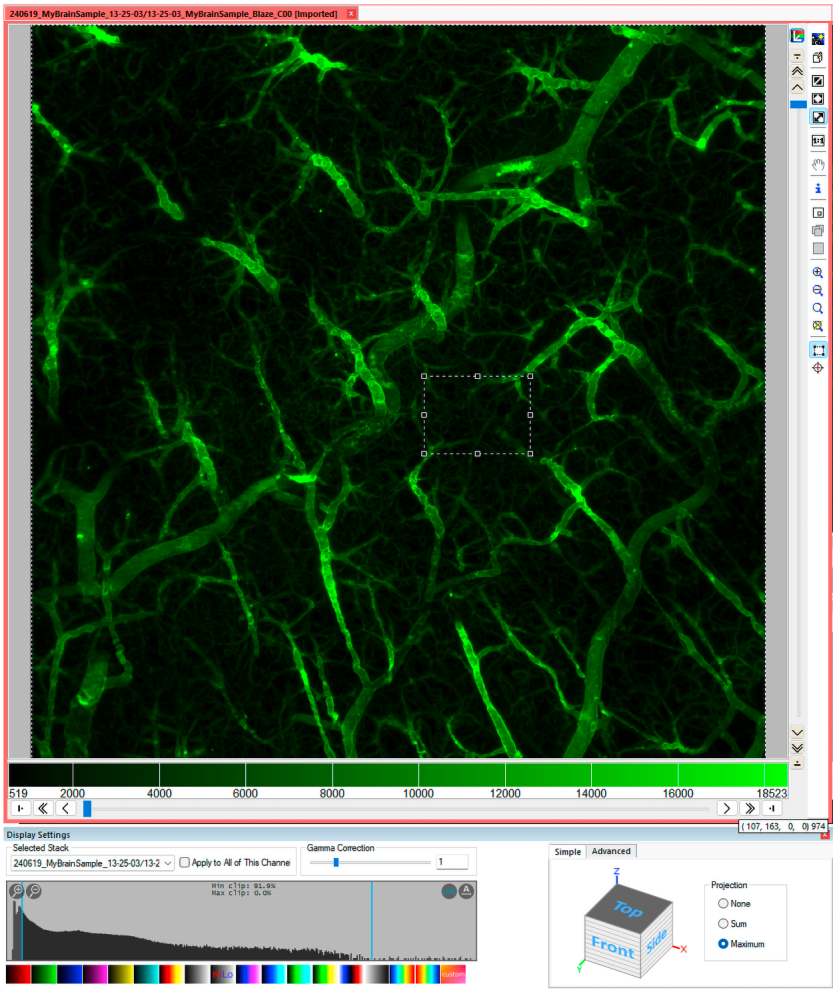


Figure 7.4: Simple view

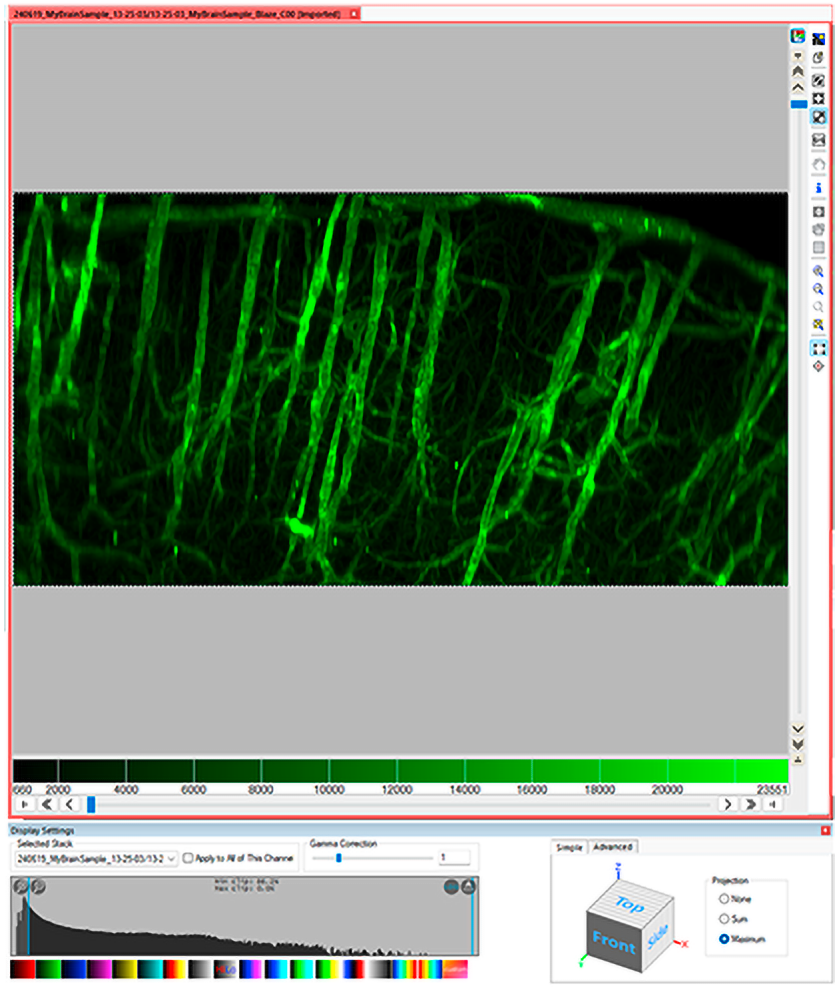
You can click one of the cube sides to change the view.

To view the data from the top:



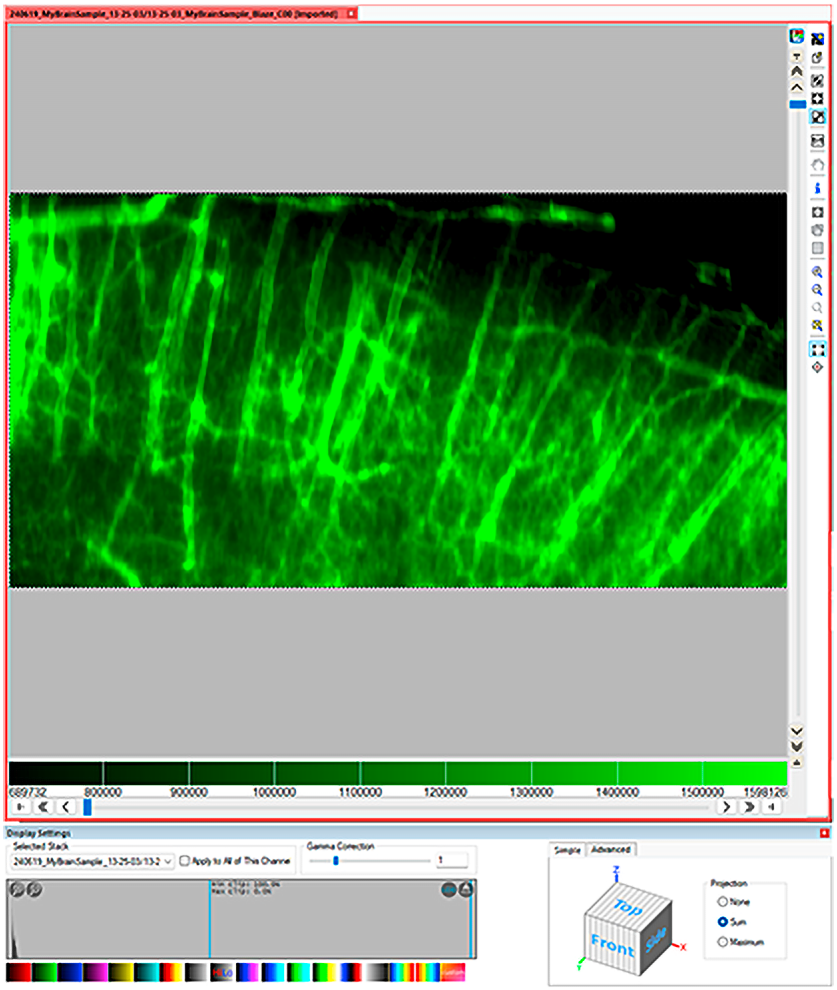
1 Click **Top**. Use the scroll bar on the right in the stack window to scroll along the z-axis from top to bottom through the data.

To view the data from the front:



1 Click **Front**. Use the scroll bar on the right in the stack window to scroll along the y-axis from front to back through the data.

To view the data from the side:



1 Click **Side**. Use the scroll bar on the right in the document window to scroll along the x-axis through the data.

A projection can be calculated for each view. By default, **Projection > None** is set, so not any projection is calculated:

- Click **Projection > Sum** to calculate a projection that sums up all pixels along an axis.

or

- Click **Projection > Maximum** to calculate a projection that uses the maximum value for a pixel along the axis.

7.4.3 Advanced view

The **Advanced** tab shows a summary of all used projections. You can change the projections by clicking them in the list.

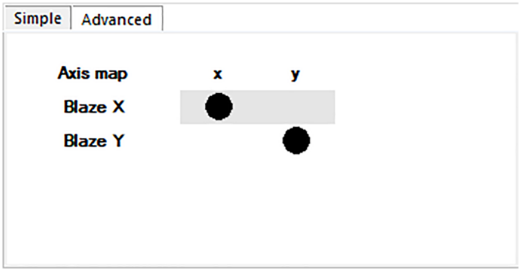


Figure 7.5: Advanced view

7.5 Merging and splitting

In the default view, the ImSpector software shows data stacks in **Gallery Mode**. In this mode, data stacks are shown side by side.

To create an overlay of multiple data stacks:

1 Right-click the image and select **Manage Channels > Merge Stacks**.

To split a data stack along an axis into a single layer stack:

1 Right-click the image and select **Manage Channels > Split Projected Axis**.

7.6 Defining a region of interest (ROI)

The ImSpector software lets you select parts of the data in the stack window. This ROI can be used for various tasks such as copying data, creating profiles, or adjusting colormaps. The pixel positions and the physical size of the ROI are shown.

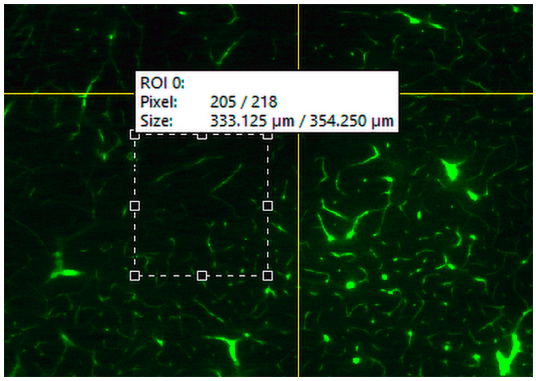


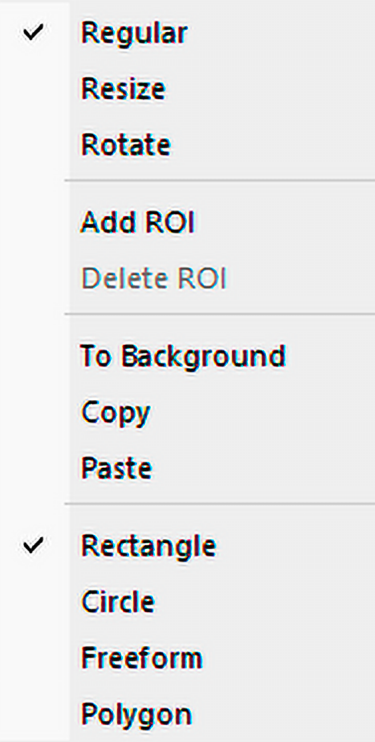
Figure 7.6: Region of interest

To define a ROI:



1 Ensure that the **ROI for Active Profile** button is activated.

2 Right-click the handles on the ROI to open a menu that allows you to customize the shape between rectangle, circle, freeform, and polygon.





3 Click the **Set as new ROI** button to apply the settings.

7.7 Showing measurement information

The ImSpector software can show various metadata of your measurement.

The following metadata can be processed:

- Instrument information (e.g., laser settings, camera settings, and objectives used)
- Measurement information (e.g., creation date and instrument mode)
- Stack information (e.g., pixel number and physical size)
- User-specific data (e.g., name and institution)
- Comments of the measurement

To show measurement information:



1 Click the **Show Measurement Information** button on the right in the stack window.

To edit comments:

1 Click **Edit comment**. A free text field opens.

2 Enter any text.

8 Data management

This chapter describes how to open, save, and export data.

8.1 Types of windows

Depending on the type of data, the ImSpector software uses different types of windows:

- Stack windows are used for measurements, viewing and analyzing data. A stack window can have several document windows in a tab structure as follows: a green tab with access to the instrument or a gray tab without. Also see **Stack window on page 45**.
- Profile windows are used to show graphs from evaluation functions.
- Stack windows opened by the **Image Series Viewer** are similar to generic stack windows. Yet all data axis handling actions can be performed in the **Display Settings** dialog. This window has a red tab.

8.2 Managing files

The stack window is always located on the right-hand side of the ImSpector software.

If you have more than one document open, you can either have them in separate windows or in a tab structure. To switch between these views, activate or deactivate **Window > Show Document Windows in Tabs** in the menu bar.

If you right-click the tab of the stack window, you can select from the following options:

- Open a new stack window or a new graph window
- Close a document or a window
- Save a document
- Export a document

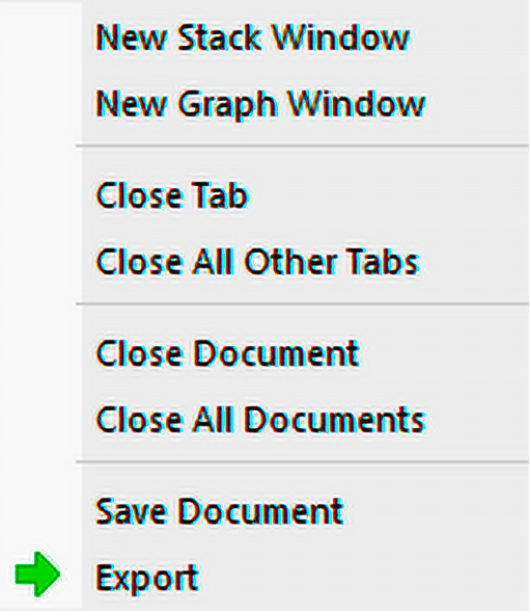


Figure 8.1: Stack window options

If you right-click a document that has no hardware access (gray tab), the option **Activate Hardware** is also available.

8.2.1 New documents

New documents can be created via **File > New** in the menu bar. See **File menu on page 14**. The new documents can be created with the current settings and the default settings.

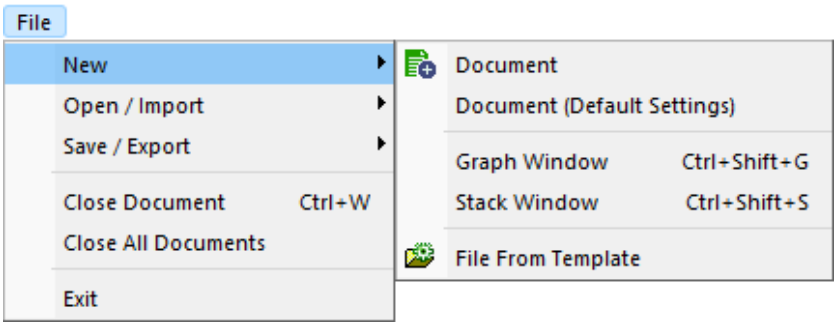


Figure 8.2: File menu

8.2.2 New graph or stack windows

New graph windows or new stack windows can be created via **File > New** in the menu bar. See **File menu on page 14**. The new windows show TIF files.

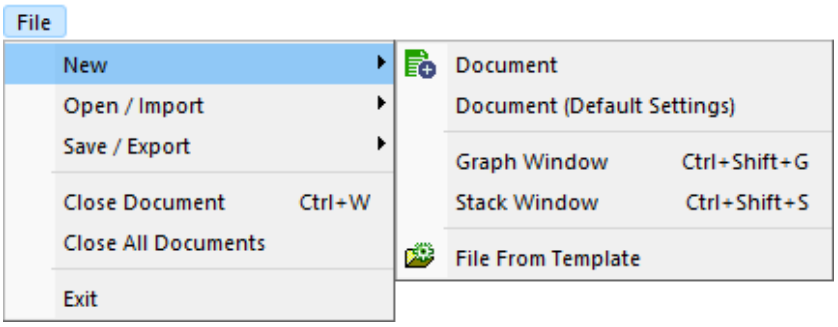


Figure 8.3: File menu

8.2.3 Open documents

Existing documents can be opened or imported via **File > Open / Import** in the menu bar. See **File menu on page 14**. Files with the extension .msr can be opened. Data saved via the **Autosave** function can be imported with the **Image Series Viewer**.

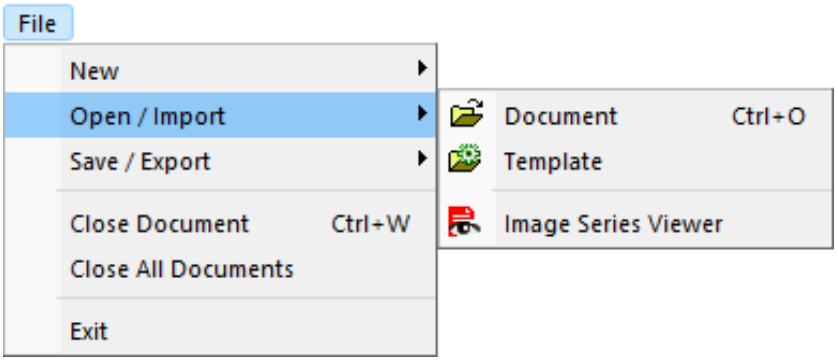


Figure 8.4: File menu

8.2.4 Save documents

Documents can be saved or exported via **File > Save / Export** in the menu bar. See **File menu on page 14**.

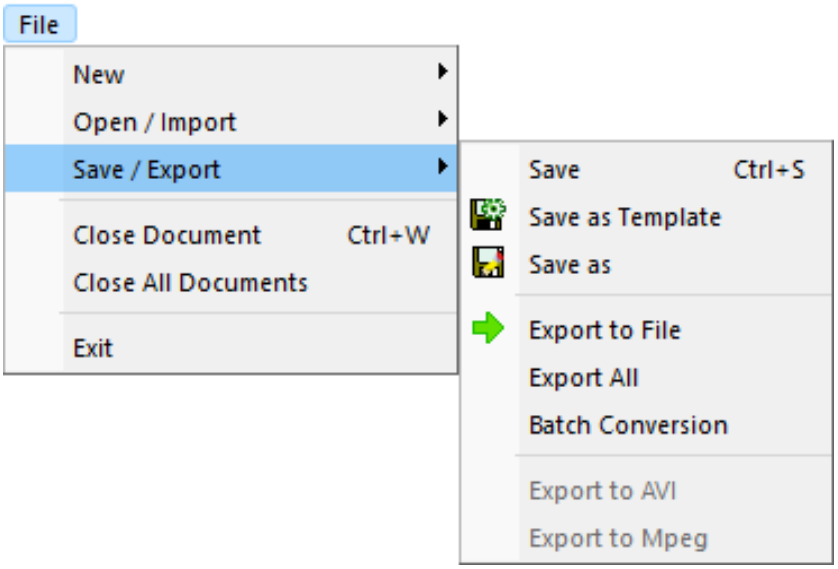


Figure 8.5: File menu

The ImSpector software saves data as SPV files and measurements as MSR files.

MSR files can be exported to different file formats, for example:

- TIFF, OME-TIFF
- RGB TIFF
- Binary Double Files (image data without any other file information)
- ASCII Data Files
- Postscript Files

To export an open file as a TIFF file:

1. Select the document to export.
2. Select **File > Save / Export > Export to File** or **File > Save / Export > Export All**.
3. Select the color depth **8 Bit** or **16 Bit** under **Data Type**.
4. Optional: Select the **Scale data** checkbox to avoid data truncation errors.
5. Optional: Clear the **Use .ome.tiff Extension** checkbox. If OME-TIFF is not used, the third and/or fourth dimension data axes can be split into single TIFF files.
6. Select either **Multi Channel** or **Single Channel** to save the TIFF files for all channels or for the selected channel only.

9 Settings

9.1 User settings

To add, remove, or change a user, you can access the user settings by clicking the corresponding icon on the toolbar.

9.2 Hardware settings

The access to the hardware settings under **Settings** > **Hardware** is restricted.

Changing hardware settings must only be performed by Miltenyi Biotec authorized service providers.

10 Important keyboard shortcuts

The ImSpector software has keyboard shortcuts to help the experienced user to use the software as efficiently as possible. The most important shortcuts are summarized in the following table.

Function	Keyboard shortcut
Main Application	
Shows or hides **Live Dialogs**	Tab
Toggles Overview mode	Shift + Tab
Shows or hides the **Image Series Viewer**	Ctrl + I
Shows or hides **Display Settings**	Alt + C
Creates a new stack window	Ctrl + Shift + S
Creates a new graph window	Ctrl + Shift + G
Opens the software documentation	F1
Full View mode	F5
Image Data	
Adjusts the contrast of the colormap automatically to the minimum pixel value in stack window	F9
Adjusts the contrast of the colormap automatically to the maximum pixel value in stack window	F10
Applies colormap to all tiles	Ctrl + Q
Toggles fixed colormap scaling	Ctrl + K
Toggles Snail mode	F3
Toggles overview MIP	F4
Zooms in	Ctrl + plus sign key (+) or mouse wheel
Zooms out	Ctrl + minus sign key (-) or mouse wheel
Copies colormap settings	Ctrl + Alt + C
Pastes colormap settings	Ctrl + Alt + V
Toggles channel overlay	Ctrl + G
Shows data stacks in one to nine gallery columns	Ctrl + 1–9

Function	Keyboard shortcut
Moves through ROI history	Ctrl + ↑/↓
Shows data stack properties	Ctrl + T
Pans the image	Spacebar + left mouse button
Adjusts the maximum level in graph window (Fit best)	F9
Adjusts the minimum level in graph window (Grow)	F10

Table 10.1: Important keyboard shortcuts

11 Technical support

For technical support, contact your local Miltenyi Biotec representative or Miltenyi Biotec Technical Support at Miltenyi Biotec headquarters:

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Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone: +49 2204 8306 3803
technicalsupport@miltenyi.com

Visit **www.miltenyibiotec.com** for local Miltenyi Biotec Technical Support contact information.

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